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Phylogenetic analysis within Monhysteridae and Thoracostomopsidae based on rDNA sequences and two new species from the Yellow Sea, China

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Abstract

Phylogenetic relationships based on rDNA in nematodes are complex and remain understudied. During our taxonomic study on Changdao Island, three species—*Thalassomonhystera tortospicula* **sp. nov.**, *Enoploides parakoreanus* **sp. nov.**, and *Enoplolaimus lenunculus* Wieser, 1959—are described. *Thalassomonhystera tortospicula* **sp. nov.** is characterized by a medium-sized body, short outer labial and cephalic setae, amphidial fovea positioned at the buccal cavity, twisted spicules, gubernaculum with a dorsocaudally directed apophysis, and a conical-cylindrical tail. *Enoploides parakoreanus* **sp. nov.** is characterized by a medium-sized body, a wide buccal cavity with three equally sized teeth, Y-shaped mandibles with claw-like distal ends, short and slightly curved spicules, a sleeve-shaped gubernaculum, a tubular-shaped precloacal supplement, and a conical-cylindrical tail. Phylogenetic analysis within Monhysteridae shows that the three morphologically similar genera—*Geomonhystera*, *Halomonhystera*, and *Monhystera*—fall into different clades, and ecological environment might be an important factor in their delineation. Phylogenetic analysis within Thoracostomopsidae shows that the genera *Enoploides*, *Epacanthion*, *Paramesacanthion*, and *Mesacanthion* each form distinct monophyletic groups. Species of *Enoplolaimus* are shown in different clades, and the phylogenetic position at the subfamily level is not well supported with the current data.

Key Words

Free-living nematode, new species, phylogenetic analysis, taxonomy

Introduction

Nematodes might be the most diverse group in the animal kingdom, with the estimated number of species ranging from half a million to 10 million (Hodda 2022). Convergent evolution and the scarcity of diagnostic characters for higher-level classification render it one of the most challenging groups in taxonomy (Leduc et al. 2017). To date, fewer than 7,000 marine species have been accepted as valid, and most of them are mainly based on morphological characters. Phylogenetic analysis based on molecular sequences, especially small subunit (SSU) and large subunit (LSU) rDNA sequences, has changed our understanding of nematode evolution despite the existence of paraphyletic and polyphyletic groups (De Ley and Blaxter 2004; Ahmed and Holovachov 2021). SSU rDNA is commonly used to resolve relationships at the family and order levels, whereas LSU rDNA is used to resolve relationships at lower taxonomic levels (De Ley et al. 2005; Leduc et al. 2017). However, relationship analyses based on SSU sometimes contradict those deduced from LSU (Guo et al. 2023a; Liang et al. 2024), which makes the complex relationships

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within nematodes even more confusing. Phylogenetic analysis based on combined rDNA sequences might reveal more detailed relationships among nematodes.

Family Monhysteridae is a widely distributed taxon occurring in marine, freshwater, and terrestrial habitats. Monhysteridae is characterized by the holapomorphy of the reproductive system being located to the right of the intestine; males have a single testis (one or two testes in *Testudinema* Abebe, Sharma, Mundo-Ocampo & Platt, 2012), and females have an outstretched, monodelphic, and prodelphic ovary (Fonseca and Bezerra 2013). Monhysterid nematodes are mostly free-living; some are commensal, and only *Halomonhystera parasitica* Poinar, Duarte & João Santos, 2010 is parasitic. Lorenzen (1978) first reviewed the family Monhysteridae systematically, alongside Xyalidae and Sphaerolaimidae. Later, Andrássy (1981) and Coomans and Eyualem-Abebe (2006) reviewed freshwater and terrestrial monhysterids, and Fonseca and Decraemer (2008) reviewed the marine monhysterids. With the addition of *Testudinema*, 18 genera have been accepted as valid. According to Jacobs (1987), Monhysteridae was subdivided into two subfamilies, Monhysterinae and Diplolaimellinae, based on buccal cavity shape, and each subfamily was further divided into two tribes, a classification that is widely accepted (Jacobs 1987; Fonseca and Decraemer 2008). Phylogenetic relationships among Monhysteridae were analyzed using cladistic analysis based on forty-nine morphological characters by Nicholas and Trueman (2002), and based on molecular sequences by Tchesunov et al. (2015) and Westerman et al. (2022).

Family Thoracostomopsidae is distributed in marine and freshwater habitats and is characterized by the holapomorphy of dorsal lateral orthometanemes. Thoracostomopsidae consists of three subfamilies—Thoracostomopsinae, Trileptiinae, and Enoplolaiminae—which are mainly distinguished according to mandible shape in the buccal cavity. Smol et al. (2014) considered *Hyptiolaimus* Cobb, 1930 invalid because the original description was based on a single juvenile. Recently, Souza and Maria (2023) reviewed Thoracostomopsidae, provided an overview of 16 genera, and considered *Fenestrolaimus* Filipjev, 1927, *Metenoploides* Wieser, 1953, and *Saveljevia* Filipjev, 1927 invalid due to the absence of male specimens. They also treated 47 species based only on juvenile and female specimens as *species inquirendae*, and identified three species as *nomina nuda*. Tchesunov et al. (2023) also discussed the position of *Marimermis maritima* Rubzov & Platonova, 1974 and considered its phylogenetic affinity with Thoracostomopsidae. Phylogenetic relationships based on molecular sequences have been analyzed by Smythe (2015), Jeong et al. (2020), and Tchesunov et al. (2023).

To study the diversity and taxonomy of free-living marine nematodes on Changdao Island, undisturbed sediments have been sampled since December 2021. Marine nematodes are the dominant meiofaunal group, and more than 200 nematode species have been discovered (Guo et al. 2023b; Liang et al. 2024). In this article, two new species—*Thalassomonhystera tortospicula* sp. nov. and *Enoploides parakoreanus* sp. nov.—are described. Ribosomal DNA sequences of LSU and SSU from *Enoploides parakoreanus* sp. nov. and *Enoplolaimus lenunculus*, as well as the SSU sequence from *Thalassomonhystera tortospicula* sp. nov., were obtained, and phylogenetic relationships within the families Monhysteridae and Thoracostomopsidae are analyzed.

Materials and methods

Specimen collection and preparation

Undisturbed sediment samples were collected from the sublittoral region of Changdao Island using a syringe with a 2.9 cm inner diameter, to a depth of 8 cm. Samples were vertically subdivided into 0–2 cm and 2–8 cm layers and fixed with 10% formalin in seawater for morphological analysis and with 95% ethanol for molecular analysis. In the laboratory, sediment samples were stained with Rose Bengal and rinsed through two sieves with mesh sizes of 500 μm and 45 μm to separate meiofauna from macrofauna (>500 μm). Heavier sediment particles were removed by centrifugation in Ludox-TM (Sigma-Aldrich Co., USA), and the meiofauna was transferred to a grid-lined Petri dish and sorted under a stereoscopic microscope. Nematodes were transferred into a mixture of ethanol (5%) and glycerin (5%), with the ethanol slowly evaporated. Nematodes were subsequently mounted in glycerin on permanent slides. Observations and measurements were made using an Axiscope 5 differential interference contrast microscope (Zeiss, Germany). Line drawings were created with ZEN 3.7 Labscope software using an iPad (Apple, USA). The type specimens were deposited at the Institute of Oceanology, Chinese Academy of Sciences, Qingdao.

Samples used for molecular analysis were treated in the same way as the formalin-fixed samples. Four male specimens of *Thalassomonhystera tortospicula* sp. nov., one male specimen of *Enoploides parakoreanus* sp. nov., and one male specimen of *Enoplolaimus lenunculus* were sorted. Identification was confirmed primarily based on buccal cavity shape, mandible shape, cephalic setae, spicule and gubernaculum shape, and tail morphology on temporary slides.

DNA extraction, PCR amplification, and phylogenetic inference

Genomic DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen, Germany) and used as a template for SSU and LSU amplification. Primers G18S4F (5'-GCT TGT CTC AAA GAT TAA GCC-3') and 18PR (5'-TGA TCC WMC RGC AGG TTC AC-3') were used for nearly full-length SSU (Blaxter et al. 1998), and primers D2A (5'-ACA AGT ACC GTG AGG GAA AGT TG-3') and D3B (5'-TCG GAA GGA ACC AGC TAC TA-3') were used for the D2–D3 fragment of LSU (Nunn 1992). PCR was conducted as described by Zhao et al. (2015). PCR products were sequenced by Genewiz (China) and assembled using

Geneious v. 6.1.2. The newly obtained SSU sequences (accession numbers: *T. tortospicula* sp. nov., PQ642907; *E. parakoreanus* sp. nov., PQ404817; *Enoplolaimus lenunculus*, PQ404814) and D2–D3 LSU sequences (*E. parakoreanus* sp. nov., PQ404815; *Enoplolaimus lenunculus*, PQ404816) have been deposited in GenBank.

SSU sequences longer than 600 bp from family Monhysteridae were retrieved from GenBank. In total, 58 sequences from 11 genera were used for phylogenetic analysis (Table 1). Sequences were aligned using the MUSCLE algorithm. Substitution models (TN93 [Tamura-Nei] + G [gamma distribution]) were selected. The analysis was rooted with *Chromadorina communis* Guo, Wang, Li & Wang, 2023 (accession number OP680597). Maximum likelihood (ML) analysis was performed using MEGA X with 1,000 bootstrap replicates. Bayesian inference (BI) was conducted using CIPRES and MrBayes v. 3.2.7a with a chain length of 10,000,000 and a burn-in fraction of 0.25. The topologies of the resulting trees were visualized using FigTree v. 1.4.3 and refined with PowerPoint 2021.

Table 1. Sequences information of SSU used in the phylogenetic analysis of the family Monhysteridae.

Species	SSU accession number	Specimen voucher	Habitat
Chromadorina communis	OP680597	22YTCD	marine
Diplolaimella sp.	MK121964	-	-
Diplolaimella ariakensis	LC625777	S3	marine
Diplolaimella ariakensis	LC625779	S2	marine
Diplolaimella dievengatensis	AM748763	-	marine
Diplolaimella dievengatensis	AJ966482	Moens T. (Ghent)	marine
Diplolaimella dievengatensis	MN072928	-	-
Diplolaimelloides delyi	ON713561	49AM10K15	brackish
Diplolaimelloides delyi	AM748762	-	terrestrial
Diplolaimelloides cf. *meyli*	ON713661	51AM3E16	brackish
Diplolaimelloides cf. *meyli*	ON713667	8AM14C16	brackish
Diplolaimelloides cf. *meyli*	ON713671	5AM5E16	brackish
Diplolaimelloides meyli	ON713672	98AM15L15	brackish
Diplolaimelloides meyli	ON713748	8AM04K15	brackish
Diplolaimelloides meyli	AF036611	-	-
Diplolaimelloides meyli	ON713580	103AM16L15	brackish
Diplolaimelloides oschei	AM748761	-	terrestrial
Eumonhystera filiformis	KJ636219	-	-
Eumonhystera filiformis	KJ636238	-	-
Eumonhystera cf. *filiformis*	KJ636240	-	-
Eumonhystera cf. *hungarica*	KJ636237	-	-
Eumonhystera cf. *longicaudatula*	KJ636252	-	-
Eumonhystera cf. *similis*	AY284691	-	terrestrial
Eumonhystera cf. *simplex*	AY284692	-	terrestrial
Eumonhystera cf. *vulgaris*	KJ636250	-	-
Eumonhystera sp.	ON713707	79AM6E16	brackish
Eumonhystera sp.	MN082286	-	-
Eumonhystera sp.	KJ636251	-	-
Gammarinema scyllae	MZ274172	T81 1	commensal
Geomonhystera villosa	LC457671	-	terrestrial
Geomonhystera villosa	EF591334	-	terrestrial
Geomonhystera sp.	LC382087	-	terrestrial
Geomonhystera sp.	KJ636213	-	-
Geomonhystera sp.	FJ040465	-	-
Geomonhystera sp.	GQ503077	NE461V	terrestrial
Monhystrium mangrovi	MZ274173	T71 1	commensal
Monhystrium mangrovi	MZ274174	T71 2	commensal
Tridentulus sp.*	AJ966507	-	marine
Tridentulus sp.*	MF542306	-	-

* This genus has been transferred to the genus *Tridentula* Andrássy, 2007.

SSU sequences longer than 600 bp and D2–D3 fragments of LSU from the family Thoracostomopsidae and *Marimermis maritima* were retrieved from GenBank. LSU and SSU sequences of *Trileptium ribeirensis* were extracted from its whole genome sequence (ASM3024822v1) using BLAST-based identification. Thirty SSU and LSU sequences derived from the same specimen, along with 22 additional SSU sequences, were used for phylogenetic analysis (Table 2) and rooted with *Viscosia dossena* Leduc & Zhao, 2023. Alignment was performed using MAFFT v. 7. A dataset of concatenated LSU + SSU sequences was constructed with PhyloSuite v. 1.2.3. Substitution models (GTR [general time-reversible] + G [gamma distribution] + I [proportion of invariable sites]) were selected for both maximum-likelihood (ML) and Bayesian inference (BI) analyses. ML and BI tree construction and refinement were conducted as in the Monhysteridae analysis.

Abbreviations are as follows: **a**, body length / maximum body diameter; **a.b.d.**, anal body diameter; **b**, body length / pharynx length; **c**, body length / tail length; **c.b.d.**, corresponding body diameter; **c′**, tail length / a.b.d; V%, position of vulva from anterior end expressed as a percentage of total body length.

Results and discussion

Morphological characterization

Systematic of *Thalassomonhystera tortospicula* sp. nov.

Family Monhysteridae de Man, 1876
Genus *Thalassomonhystera* Jacobs, 1987

***Thalassomonhystera tortospicula* sp. nov.**

https://zoobank.org/197AFDB2-0106-401A-9844-FD0621AFE1D7

Figs 1, 2, Table 3

Type material. Four males and three females were collected from Station CDLJ in March 2023. ***Holotype*:** • ♂1 on slide number 23CDLJ 4-4, ***paratypes*:** • m#2, m#3, m#4 and f#1 on 23CDLJ 4-1; f#2 on 23CDLJ 4-3, f#3 on 23CDLJ 4-4.

Type locality and habitat. All specimens were collected from Changdao Island, Shandong Province, China. 37°54'N, 120°45'E (LJ), 0–2 cm sediment depth, sandy sediment.

Etymology. The specific name refers to spicules shape jointed.

Measurement. All measurement data can be found in Table 3.

Description. Male. Body medium-sized, anterior and posterior body ends narrowed. Cuticle finely striated. Inner labial sensillae papilliform. Six outer labial setae and four head setae short and in one circle. Cervical setae not found. Amphidial fovea small and circular (28–33% c.b.d.), situated at the end of buccal cavity. Cheilostoma extensive with sclerotized walls. Buccal cavity funnel-shaped. The pharynx slender, muscular, slightly expanding to its base. Nerve ring at 49–63% of pharynx length.

Table 2. Sequences information of SSU and LSU used in the phylogenetic analysis of the subfamily Thoracostomopsidae.

Species	SSU accession number	LSU accession number	Specimen voucher	Habit
Enoploides brunettii	AY854193	-	-	-
Enoploides delamarei	MG599079	-	-	-
Enoploides longispiculosus	MG669767	-	-	-
Enoploides parakoreanus sp. nov.	PQ404817	PQ404815	3-4	marine
Enoploides sp.	HM564550	HM564758	DBA2	marine
Enoploides sp.	HM564549	HM564757	DBA1	marine
Enoploides sp.	MG599077	-	-	-
Enoploides sp.	HM564448	HM564704	-	marine
Enoplolaimus attenuatus	MG669770	-	Ugent_CeMoFe17C21F_18S	-
Enoplolaimus lenunculus	PQ404814	PQ404816	3-2	marine
Enoplolaimus lenunculus	MG599050	-	-	-
Enoplolaimus lenunculus	MG599047	-	-	-
Enoplolaimus sp.	HM564473	HM564729	WUS7	marine
Enoplolaimus sp.	HM564471	HM564727	WUS5	marine
Enoplolaimus sp.	HM564467	HM564723	WUS1	marine
Enoplolaimus sp.	HM564462	HM564718	SUS15	marine
Enoplolaimus sp.	HM564461	HM564717	SUS10	marine
Enoplolaimus sp.	HM564460	HM564716	SUS1	marine
Enoplolaimus sp.	HM564434	HM564702	NAR9	marine
Epacanthion hirsutum	MG599065	-	-	-
Epacanthion longicaudatum	MG599066	-	-	-
Epacanthion longicaudatum	MG599067	-	-	-
Epacanthion sparsisetae	MG599068	-	-	-
Marimermis maritima	MZ504144	MZ504144	-	-
Mesacanthoides sp.	KR265040	-	-	marine
Mesacanthoides sp.	MG599081	-	-	-
Mesacanthion audax	MG599058	-	-	-
Mesacanthion audax	MG599059	-	-	-
Mesacanthion audax	MG599060	-	-	-
Mesacanthion audax	MG599062	-	-	-
Mesacanthion infantile	MG599051	-	-	-
Mesacanthion infantile	MG599052	-	-	-
Mesacanthion sp. *	OQ065264, OQ065170	OQ031174	A36	-
Mesacanthion sp. *	OQ065261, OQ065165	OQ031173	A15	-
Mesacanthion sp. *	OQ065259, OQ065163	OQ031172	A10	-
Mesacanthion sp. *	OQ065254, OQ065158	OQ031169	A3	-
Paramesacanthion sp.	MW258650	MW258633	SB141	-
Paramesacanthion sp.	MW258649	MW258632	SB121	-
Paramesacanthion sp.	MW258647	MW258630	SB142	-
Paramesacanthion sp.	MW258646	MW258629	SB119	-
Paramesacanthion sp.	MW258641	MW258624	SB144	-
Paramesacanthion sp.	MK007612	MK007592	SB123	-
Paramesacanthion sp.	MK007611	MK007591	SB122	-
Paramesacanthion sp.	MK007610	MK007590	SB120	-
Paramesacanthion sp.	MK007605	MK007585	KBI28	-
Paramesacanthion sp.	MK007604	MK007584	SB125	-
Paramesacanthion sp.	MK007603	MK007583	KB35	-
Paramesacanthion sp.	MK007602	MK007582	SB124	-
Thoracostomopsis barbata	MZ476006	-	-	marine
Thoracostomopsis sp.	KR265051	-	-	marine
Trileptium ribeirensis	JASANE010014375	JASANE010003111	-	-
Viscosia dossena	OK317193	OK317218	NNCNZ3371	-

* indicates that SSU sequences were aligned and assembled using Mega X.

Cardia muscular and inverse triangular-shaped, protruding into the lumen of the intestine. Excretory gland positioned posterior to the pharynx region and excretory pore posterior to nerve ring.

Single testis outstretched and located to the right of the intestine. Spicules paired and strongly curved, proximal end not cephalated and twisted at one-third from proximal end, distal end tapered, 1.5–1.6 cloacal body diameters in length. Gubernaculum with dorsal-caudal directed apophysis, 0.36–0.42 cloacal body diameters. Tail conical-cylindrical, cylindrical portion 27.6–31.2%. Three caudal glands in line with a common secretory pore.

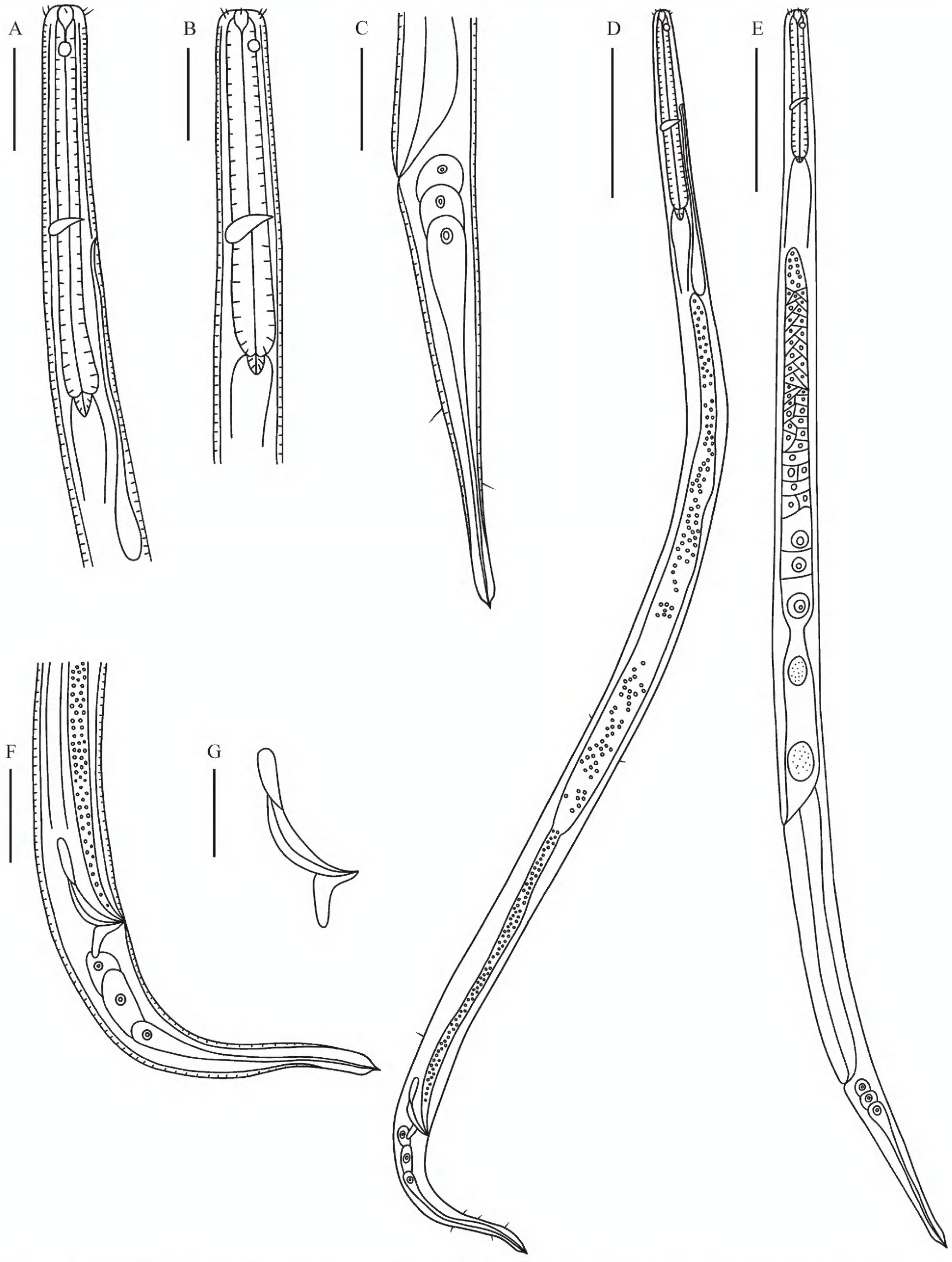

Figure 1. *Thalassomonhystera tortospicula* sp. nov. **A.** Lateral view of the male anterior region; **B.** Lateral view of the female anterior region; **C.** Lateral view of the female posterior body; **D.** Lateral view of the male entire body; **E.** Lateral view of the female entire body; **F.** Lateral view of the male posterior body, showing the tail region; **G.** Spicules and gubernaculum. Scale bars: 30 µm (**A, B, C, F**); 100 µm (**D, E**); 20 µm (**G**).

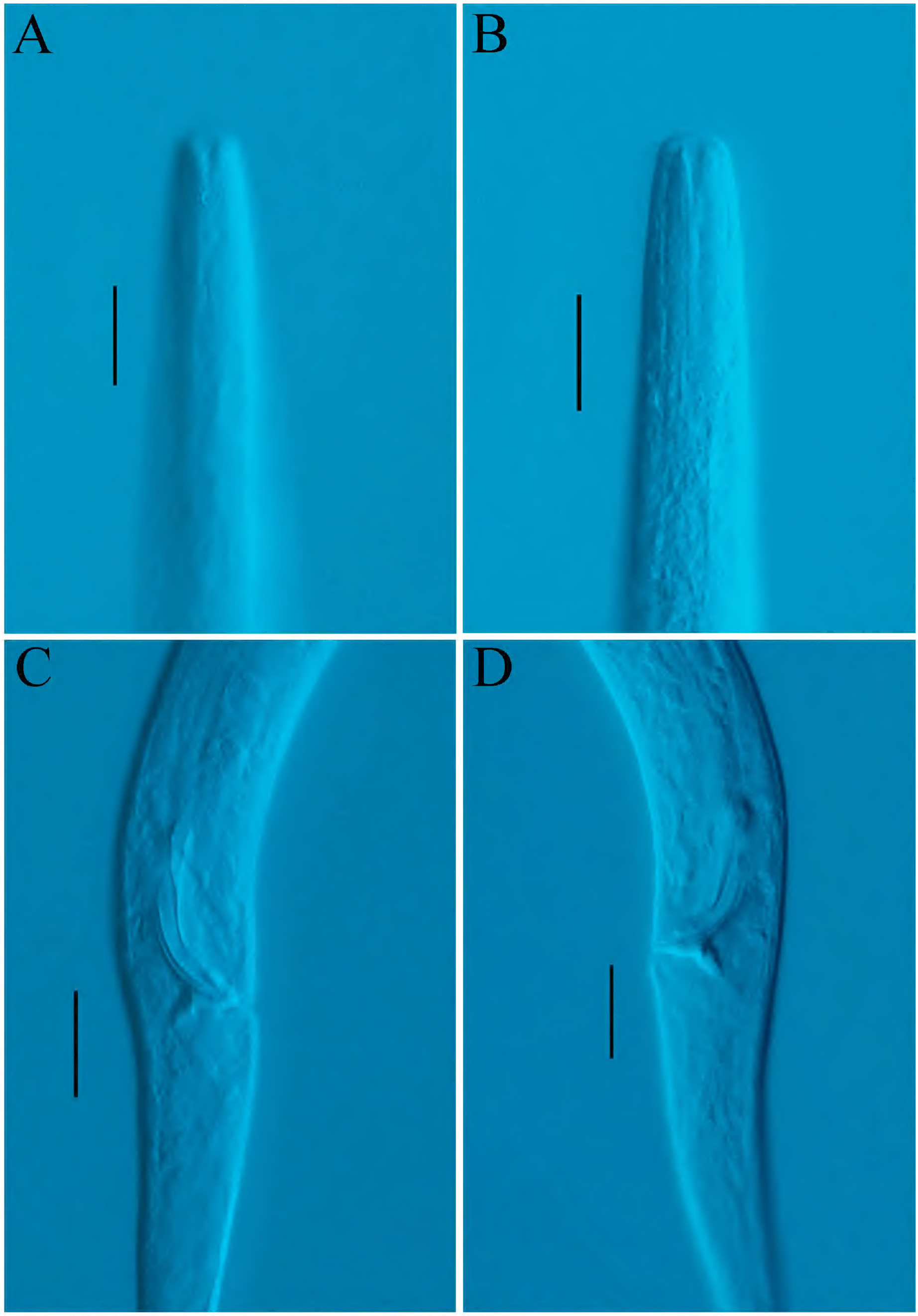

Figure 2. *Thalassomonhystera tortospicula* sp. nov. **A.** Lateral view of the male anterior region, showing amphidial fovea; **B.** Lateral view of the male anterior region, showing cephalic setae and buccal cavity; **C.** Lateral view of the male posterior region, showing spicules; **D.** Lateral view of the male posterior body, showing gubernaculum. Scale bars: 20 µm (**A–D**).

Table 3. Individual measurements of *Thalassomonhystera tortospicula* sp. nov. (in µm, except ratio).

Characters	Holotype	Paratypes	Paratypes
	male	males (n = 3)	females (n = 3)
Total body length	913	994.3 ± 39.7 (963–1039)	923 ± 35.6 (883–951)
Maximum body diameter	28	32 ± 1 (31–33)	34 ± 1 (33–35)
Head diameter	11	11.7 ± 0.6 (11–12)	12.3 ± 1.2 (11–13)
Length of outer labial setae	4	4 ± 0 (4–4)	3.9 ± 0.2 (3.7–4)
Length of cephalic setae	3	3.3 ± 0.3 (3–4)	3.1 ± 0.4 (3–4)
Buccal cavity depth	6	7.3 ± 1.2 (6–8)	7.3 ± 1.2 (6–8)
Amphidial fovea width	4	4.3 ± 0.6 (4–5)	3.5 ± 0.2 (3–4)
Amphidial fovea from anterior	12	10.7 ± 0.6 (10–11)	16.7 ± 1.2 (10–11)
Body diameter at amphidial fovea	14	15 ± 0 (15–15)	10.7 ± 0.6 (16–18)
Nerve ring from anterior	64	81.7 ± 4 (77–84)	80 ± 1 (79–81)
Body diameter at nerve ring	19	21.7 ± 0.6 (21–22)	23 ± 0 (23–23)
Pharynx length	130	134 ± 1 (133–135)	131.3 ± 1.5 (130–133)
Body diameter at base of pharynx	21	23 ± 0 (23–23)	23 ± 0 (23–23)
Excretory pore from anterior	97	90 ± 5.3 (86–89)	-
Anal body diameter	24	26 ± 1 (25–27)	21.3 ± 0.6 (21–22)
Spicules length along arc	39	40.3 ± 1.5 (39-42)	-
Gubernaculum length	10	10 ± 1 (9–11)	-
Vulva from anterior	-	-	582.7 ± 21.5 (562–605)
Body diameter at vulva	-	-	30.7 ± 0.6 (30–31)
V%	-	-	60 ± 0 (62–64)
Tail length	128	131 ± 8.9 (121–138)	128 ± 7 (123–136)
a	32.6	31.1 ± 1.7 (29.2–32.5)	27.2 ± 1.7 (25.2–28.3)
b	7	7.4 ± 0.2 (7.2–7.7)	7 ± 0.2 (6.8–7.2)
c	7.1	7.6 ± 0.5 (7.2–8.1)	7.2 ± 0.4 (6.9–7.6)
c'	5.3	5 ± 0.2 (4.8–5.3)	6 ± 0.4 (5.6–6.5)

Female. Similar to males in most respects except amphidial fovea smaller (20.6%–21.9%). Ovary outstretched and prodelphic, located to the right of intestine. Vulva located at posterior portion of the body (62–64% of the body length from the anterior end), 212–238 µm from anus.

Diagnosis. *Thalassomonhystera tortospicula* sp. nov. is characterized by body medium sized, outer and labial setae short, amphidial fovea positioned at the buccal cavity, spicules twisted, gubernaculum with dorsal-caudal directed apophysis, tail conical-cylindrical.

The new species differs from other species of *Thalassomonhystera* in the spicules anterior twisted. *Thalassomonhystera tortospicula* sp. nov. is similar to *Thalassomonhystera attenuata* Jacobs, 1987, *Thalassomonhystera lepta* Gagarin & Nguyen Dinh Tu, 2021, *Thalassomonhystera parva* (Bastian, 1865) Jacobs, 1987, *Thalassomonhystera siamensis* Kito & Aryuthaka, 1998 with amphidial fovea position, but differs from *T. attenuata* in spicules length (1.5–1.6 cloacal body diameters vs. 1.1 cloacal body diameters) and gubernaculum length (9–11 µm vs. 7 µm) (Jacobs 1987); differs from *T. lepta* in outer labial setae length (4 µm vs. 6.5–8.5 µm) and tail shape (conical-cylindrical vs. elongated conical) (Gagarin and Tu 2021); differs from *T. parva* in absence of cervical setae (present in *T. parva*), secretory pore position (posterior to nerve ring vs. anterior to nerve ring) (Jacobs 1987); differs from *T. siamensis* in body length (883–1039 µm vs. 497–655 µm), absence of cervical setae (present in *T. siamensis*), and gubernaculum apophysis shape (straight vs. curved) (Kito and Aryuthaka 1998).

Systematic of *Enoploides parakoreanus* sp. nov.

Family Thoracostomopsidae Filipjev, 1927
Genus *Enoploides* Ssaweljev, 1912

Enoploides parakoreanus sp. nov.

https://zoobank.org/73BCB7D2-BF72-426C-ABD8-0AA752B9DE8C

Figs 3–5, Table 4

Type material. One male and three females were measured and studied. ***Holotype*:** m#1 on slide 23TJGK-D-3-2-1; ***paratypes*:** f#1 on 23TJGK-X-4, f#2 on 23TJGK-D-3-1-2; f#3 on 23TJGK-D-3-1-10.

Type locality and habitat. All specimens were collected from Changdao Island, Shandong Province, China. 38°9'N, 120°44'E (TJGK), 0–8 cm sediment depth, sandy sediment.

Etymology. The specific name refers to the morphological character similar to *Enoploides koreanus*.

Measurement. All measurement data can be found in Table 4.

Description. Male. Body medium-sized and cuticle smooth. Three lips high, with its border heavily striated with grooves, each lip with two inner labial setae (0.62 head diameters in length). Six longer outer labial setae and four shorter cephalic setae in one circle, situated at anterior portion of cephalic capsule. Eight subcephalic setae at posterior end of cephalic capsule, two in each group. Buccal cavity wide and funnel-shaped, armed with three equally sized mandibles. Mandibles Y-shaped, two lateral bars each with claw-like distal end converging into one solid bar, curving inwards to

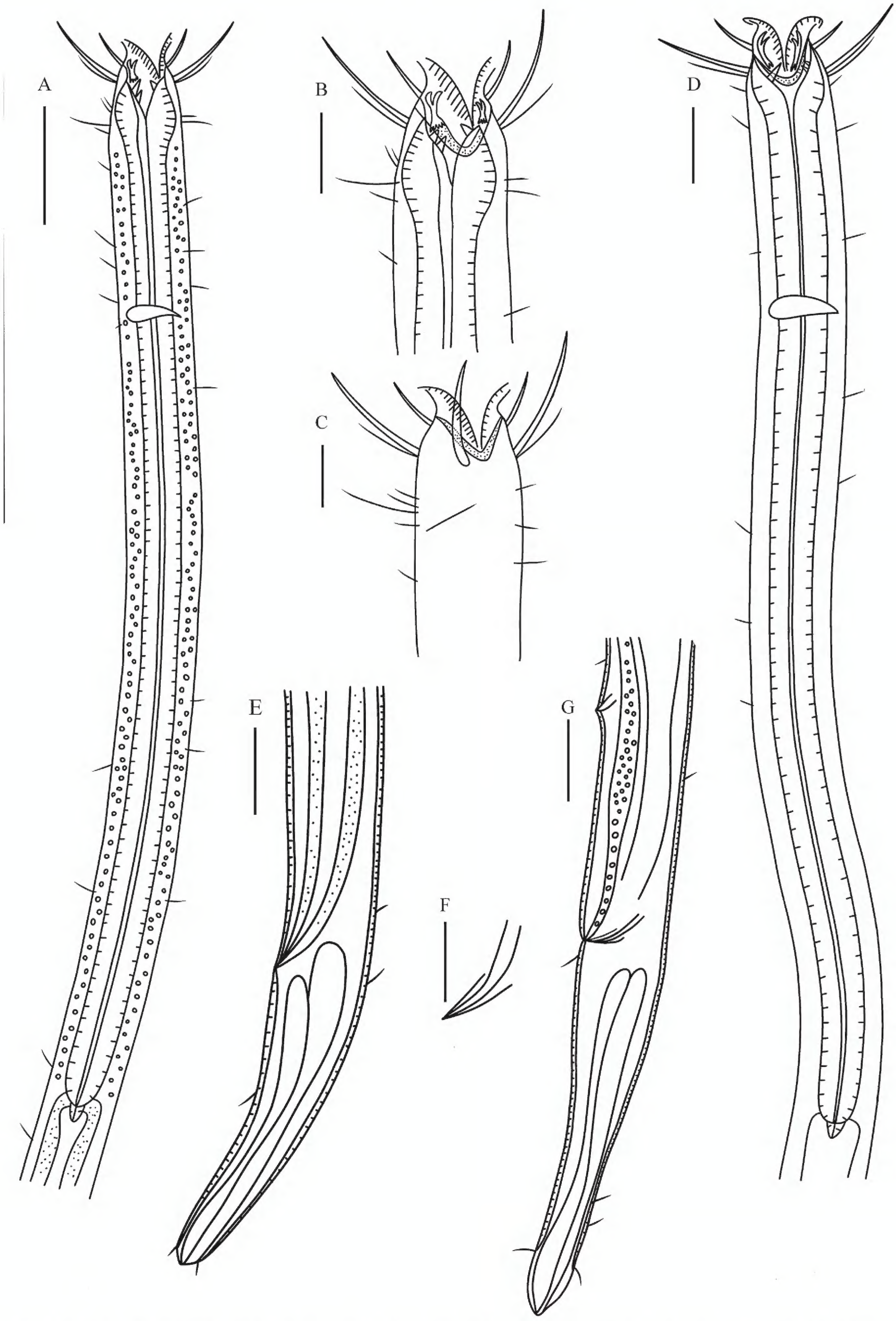

Figure 3. *Enoploides parakoreanus* sp. nov. **A.** Lateral view of the male anterior region, showing the pharynx region; **B.** Lateral view of the male anterior region, showing buccal cavity; **C.** Lateral view of the male anterior region; **D.** Lateral view of the female anterior region; **E.** Lateral view of the female posterior region; **F.** Spicules and gubernaculum; **G.** Lateral view of the male posterior body. Scale bars: 80 μm (**A**); 30 μm (**B, C, G**); 50 μm (**D, E**); 20 μm (**F**).

Figure 4. *Enoploides parakoreanus* sp. nov. **A.** Lateral view of the male entire body; **B.** Lateral view of the female entire body. Scale bars: 150 μm.

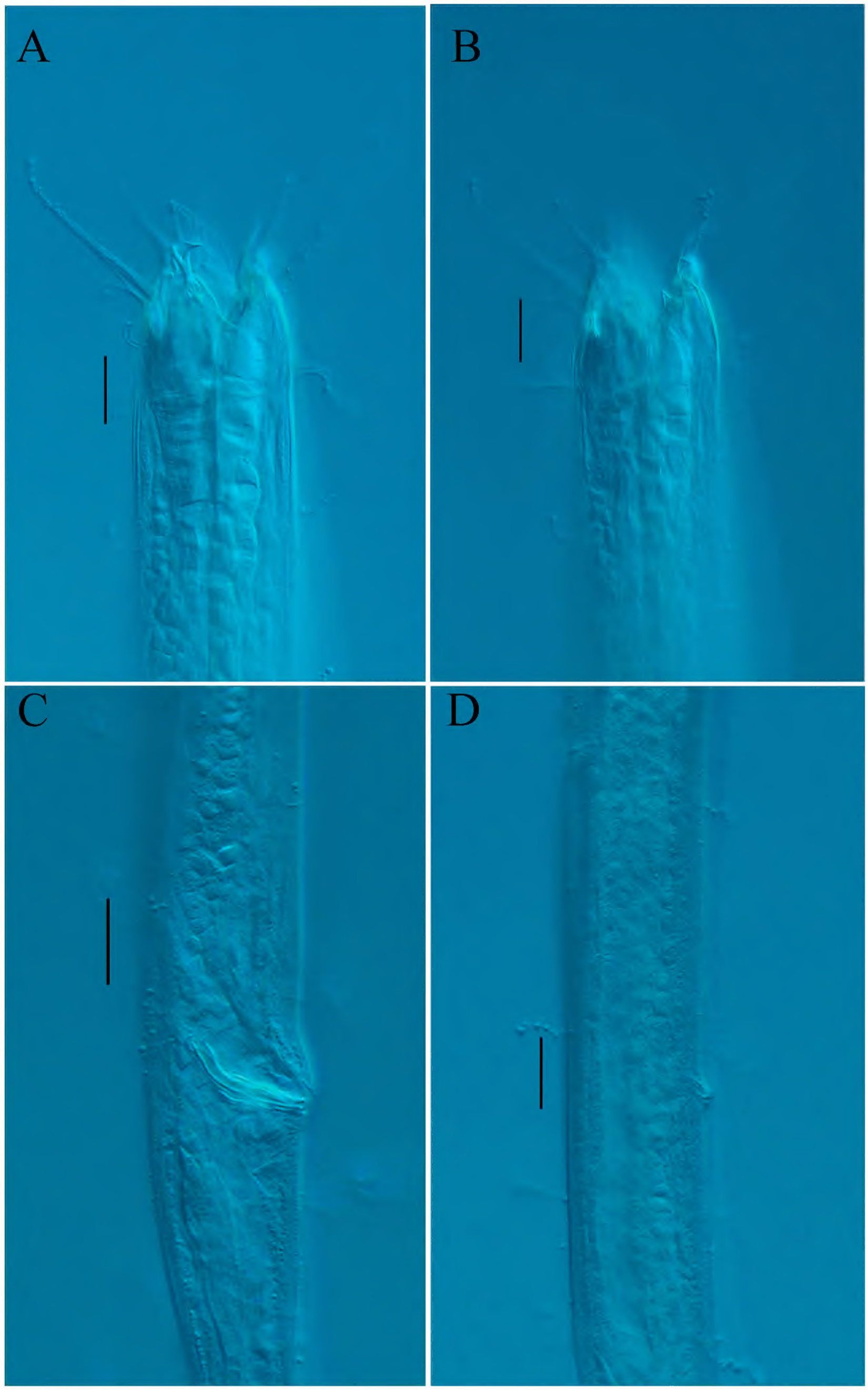

Figure 5. *Enoploides parakoreanus* sp. nov. **A.** Lateral view of the male anterior region, showing cephalic setae and mandibles; **B.** Lateral view of the male anterior region, showing tooth; **C.** Lateral view of the male posterior body, showing spicules and gubernaculum; **D.** Lateral view of the male posterior body, showing precloacal supplement. Scale bars: 20 μm (**A–D**).

Table 4. Individual measurements of *Enoploides parakoreanus* sp. nov. (in µm, except ratio).

Characters	Holotype male	Paratypes females (n = 3)
Total body length	2716	2982 ± 379.5 (2627–3382)
Maximum body diameter	48	59 ± 7.9 (53–68)
Head diameter	40	45.7 ± 5.5 (42–52)
Length of inner labial setae	22	22 ± 1 (21–23)
Length of outer labial setae	55	56.7 ± 9 (51–67)
Length of cephalic setae	19	23 ± 5.2 (20–29)
Length of subcephalic setae	32	-
Buccal cavity depth	28	30.7 ± 3.8 (28–35)
Nerve ring from anterior	165	169 ± 15.9 (157–187)
Body diameter at nerve ring	49	52 ± 4.4 (49–57)
Pharynx length	540	59 ± 7.9 (557–732)
Body diameter at the base of pharynx	48	626.3 ± 93 (53–68)
Anal/cloacal body diameter	38	44.7 ± 6.4 (40–52)
Spicules length along arc	31	-
Gubernaculum length	21	-
Vulva from anterior	-	1892 ± 257.6 (1681–2179)
Body diameter at vulva	-	57.3 ± 9.5 (50–68)
V%	-	60 ± 0 (62–64)
Tail length	153	167.3 ± 12.1 (158–181)
a	56.6	50.7 ± 4.3 (46.9–55.4)
b	5	4.8 ± 0.4 (4.5–5.3)
c	17.8	17.8 ± 1.1 (16.6–18.7)
c'	4	3.8 ± 0.3 (3.5–4.1)

the lumen. Three onchia equal-sized, posterior to each base of mandible. Amphidial fovea not observed. Pharynx long with grooves and sinuous external contours. Nerve rings anterior to middle pharynx region (30.6% of pharynx length). Secretory-excretory pore not observed. Cardia inverse triangular shaped, embedded into the intestine. Somatic setae irregularly scattered along the cervical region. Metanemes not observed.

Testes paired and outstretched, anterior and posterior testes to the right of the intestine. Spicules paired, slightly curved, 0.82 cloacal body diameters in length. Gubernaculum simple, sleeve-shaped, parallel to distal end of spicules. Precloacal supplement tubular-shaped, 8 µm in length, 105 µm from cloacal. Tail conical-cylindrical. Caudal setae present and two caudal glands with a common duct.

Female. Similar to males in most respects except subcephalic setae absent. Vulva located at posterior portion of the body (62–64% of the body length from the anterior end). Reproductive system didelphic-amphidelphic, ovaries reflexed. Vagina with thick walls and well-developed sphincter muscles.

Diagnosis. *Enoploides parakoreanus* sp. nov. is characterized by body medium-sized, mandibles Y-shaped with distal end claw-like, three equal-sized teeth, spicules paired and slightly curved, gubernaculum sleeve-shaped and enclosing the distal end of spicules, precloacal supplement tubular-shaped, tail conical-cylindrical.

Genus *Enoploides* consists of 27 valid species and can be divided into two groups by the length of spicules: the first group with short spicules (<150 µm); the second group with long spicules (>150 µm). *Enoploides parakoreanus* sp. nov. belongs to the first group. The new species differs from other species of *Enoploides* with spicules slightly curved. *Enoploides parakoreanus* sp. nov. is similar to *E. cirrhatus* Filipjev, 1918, *E. disparilis* Sergeeva, 1974, *E. koreanus* Jeong, Tchesunov & Lee, 2020, with short spicules, but differs from *E. cirrhatus* in body length (2627–3382 µm vs. 4350 µm), de Man's a values (46.9–56.6 vs. 26), and precloacal supplement (one tubular supplement vs. seven pairs of precloacal papillae) (Filipjev 1918); differs from *E. disparilis* in precloacal supplement (one supplement vs. absent), de Man's c ratio (16.6–18.7 vs. 9) (Sergeeva 1974); differs from *E. koreanus* in outer labial setae length (51–67 µm vs. 30–43 µm), spicules shape (spicules slightly curved vs. curved at obtuse angle), and gubernaculum length (21 µm vs. 12 µm) (Jeong et al. 2020).

Description of *Enoplolaimus lenunculus* Wieser, 1959

Figs 6–8, Table 5

Materials examined. Four males and three females were collected from Station TJGK in October 2023. ♂1 on slide number 23TJGK-Q-3-1-5; m#2, m#3, and f#3 on 23TJGK-Q-3-1-3; f#1 and f#2 on TJGK-Q-3-1-6.

Collection locality and habitat. All specimens were collected from Changdao Island, Shandong Province, China. 38°9'N, 120°44'E (TJGK), 0–2 cm sediment depth, sandy sediment.

Measurement. All measurement data can be found in Table 5.

Description. Male. Body long, cylindrical, slightly tapered posteriorly. Cuticle smooth. Head dome-shaped and set off with three lips, without striations. Each lip carrying a triangular lip lobe and separated by clefts, bearing two inner labial sensilla. Cephalic organ oval-shaped, just posterior to inner labial setae. Six inner labial setae long, 0.39–0.51 head diameter in length. Six outer labial setae and four cephalic setae in one circle, positioned at level of posterior margin of cephalic capsule. Rows of 8 subcephalic setae situated behind the cephalic sensilla, 15–17 µm in length. Cephalic capsule well developed. Buccal cavity conical with three equal-sized mandibles and three onchia. Mandibles well developed, each consisting of two lateral rods and one curved bar. Three onchia, one dorsal large and two ventrosublateral small. Amphidial fovea and excretory pore not observed. Pharynx cylindrical.

Male reproductive system diorchic, anterior and posterior testes outstretched. Spicules arcuated and symmetrical, 1.3–1.7 cloacal body diameters in length. Gubernaculum short. Precloacal supplement tubular-shaped, 8 µm in length, 72–82 µm from cloacal. Tail conico-cylindrical with terminal setae, 2.8–3.4 cloacal body diameters in length. Three caudal glands with a common duct.

Female. Similar to male in general body shape. Reproductive system didelphic and amphidelphic, ovaries reflexed. Vagina with thick walls. Sphincter muscles well developed, present at oviduct and posterior ovary. Vulva located at slightly posterior portion of the body (57–62% of the body length from the anterior end).

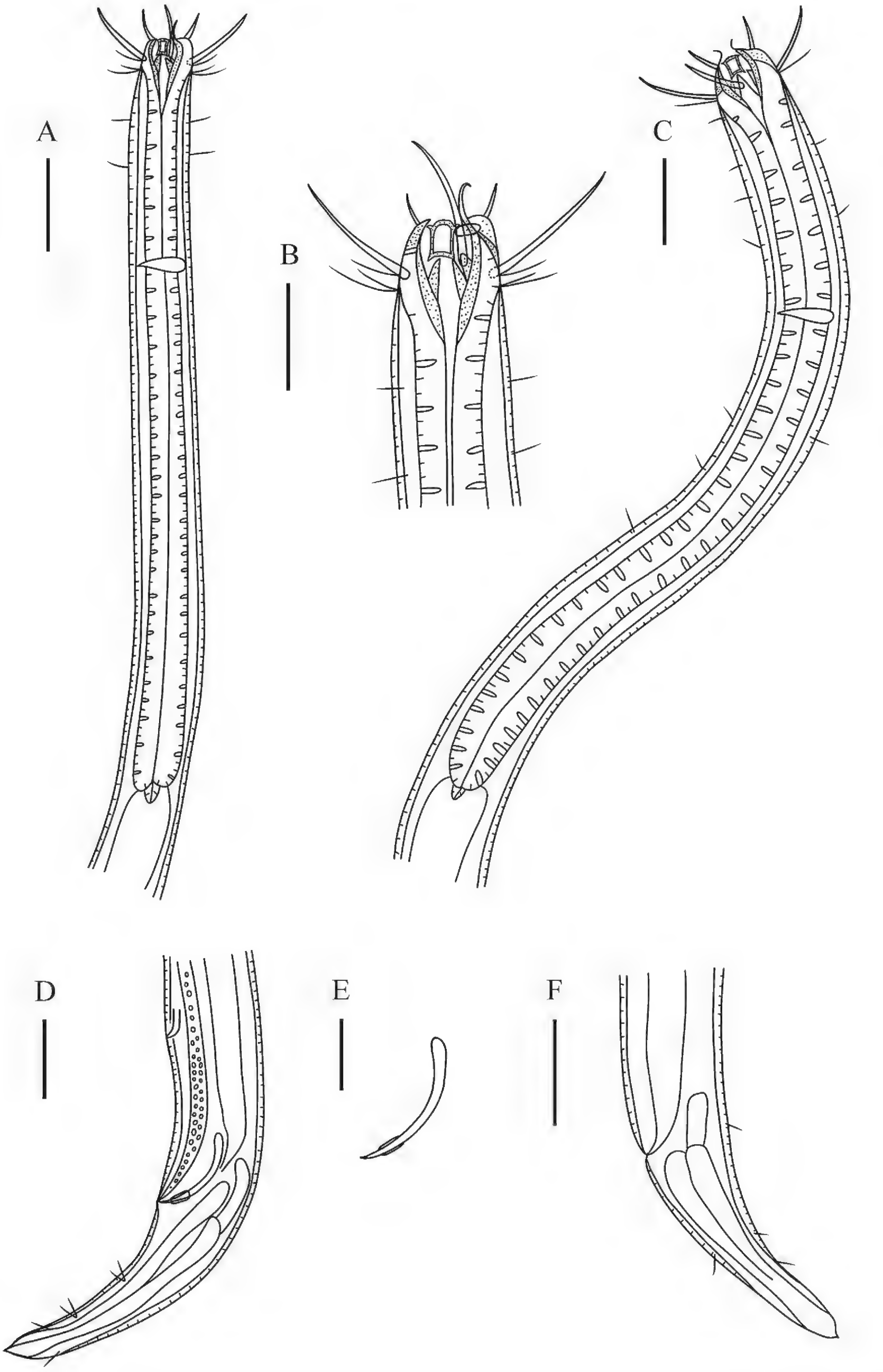

Figure 6. *Enoplolaimus lenunculus*. **A.** Lateral view of the male anterior region; **B.** Lateral view of the male anterior region showing buccal cavity, onchia, and mandibles; **C.** Lateral view of the female anterior region; **D.** Lateral view of the male posterior body; **E.** Spicules and gubernaculum; **F.** Lateral view of the female posterior body showing the tail region. Scale bars: 50 μm (**A, C, F**); 30 μm (**B, D**); 20 μm (**E**).

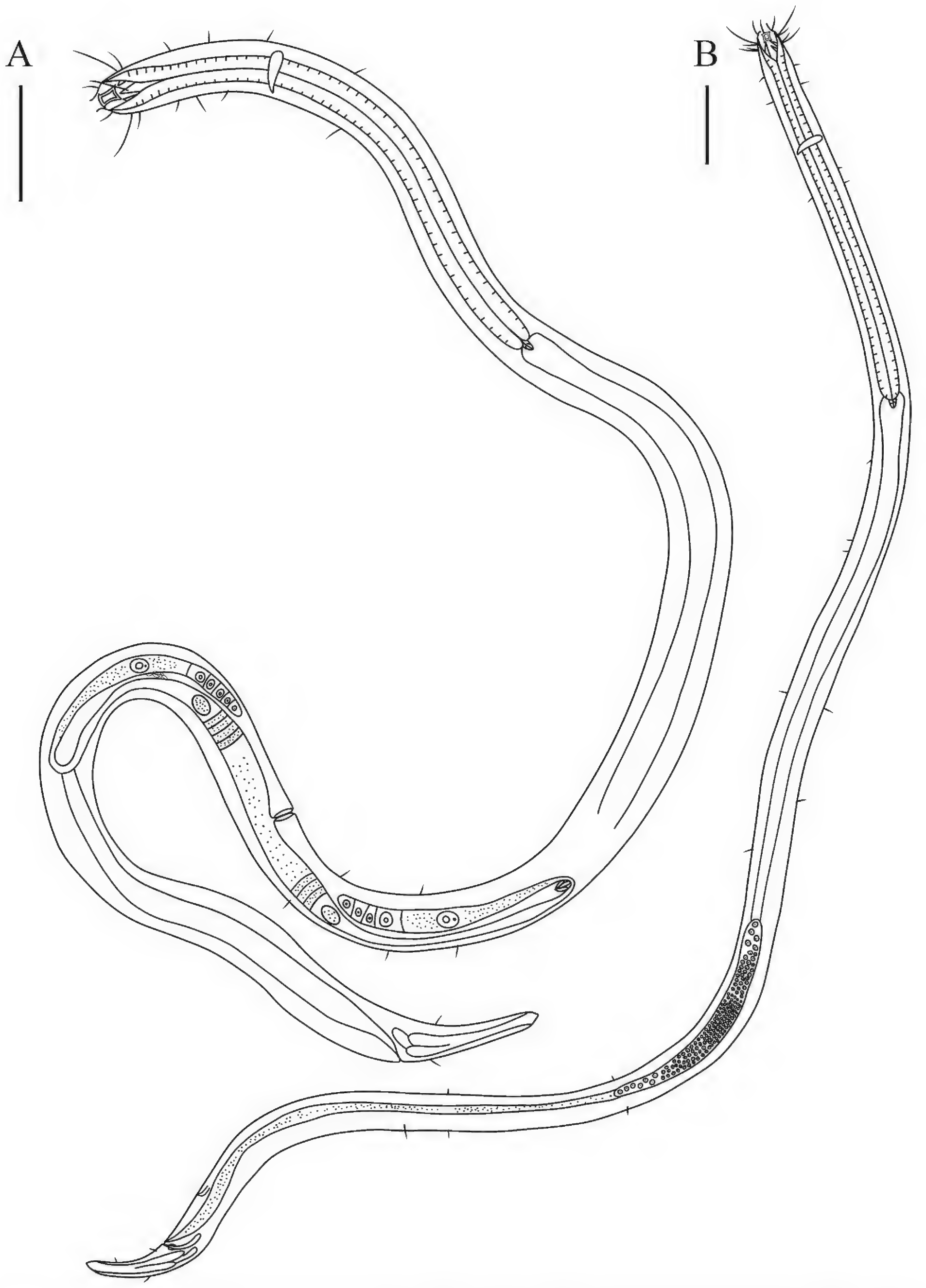

Figure 7. *Enoplolaimus lenunculus* **A.** Lateral view of the female entire body; **B.** Lateral view of the male entire body. Scale bars: 100 µm.

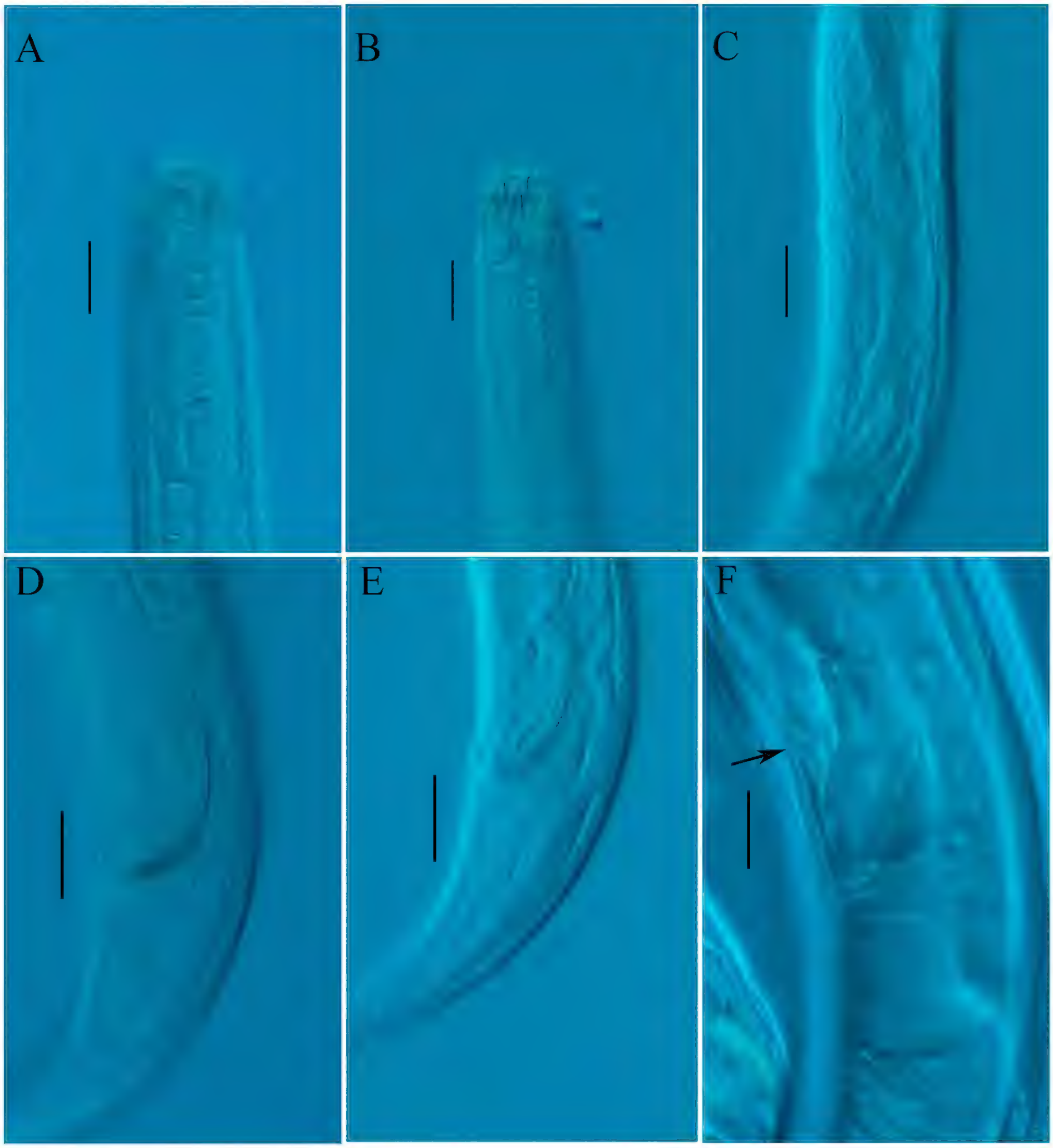

Figure 8. *Enoplolaimus lenunculus*. **A.** Lateral view of the male anterior region; **B.** Lateral view of the male anterior region showing mandibles; **C.** Lateral view of the male posterior body, showing precloacal supplement; **D.** Lateral view of the male posterior region showing spicules; **E.** Lateral view of the male posterior body, showing gubernaculum; **F.** Lateral view of the female posterior body, showing sphincter muscles (arrow). Scale bars: 20 µm (**A–F**).

Remarks. The Changdao specimens are largely consistent with the description of *E. lenunculus* by Wieser (1959) based on specimens from Bainbridge Island. However, the following discrepancies were observed between specimens from Changdao and the Bainbridge Island: subcephalic setae (absent in original description), cephalic setae length (16–23 µm vs. 12 µm), tail length (2.8–3.2 a.b.d. vs. 3.3–3.5 a.b.d.), and supplement distance from cloacal (72–82 µm vs. 90 µm).

Molecular phylogenetic relationships and analysis

The ML topology trees based on rDNA sequences are mostly identical to the BI trees, and only the BI trees are shown in Figs 9, 10. According to Nemys (2025), the family Monhysteridae includes 18 genera, and the family Thoracostomopsidae includes 16 genera. Relationships within Monhysteridae were discussed by

Table 5. Individual measurements of *Enoplolaimus lenunculus* (in µm, except ratio).

Characters	males (*n* = 4)	females (*n* = 3)
Total body length	2150.8 ± 74.5 (2057–2234)	2545 ± 177.3 (2378–2731)
Maximum body diameter	41.5 ± 1.3 (40–43)	52 ± 2.6 (49–54)
Head diameter	28.8 ± 1.7 (27–31)	38 ± 1 (37–39)
Length of inner labial setae	12.8 ± 1.3 (11–14)	14.3 ± 1.5 (13–16)
Length of outer labial setae	38.8 ± 1.3 (37–40)	45 ± 2.6 (43–48)
Length of cephalic setae	17.8 ± 2.5 (15–21)	22.7 ± 0.6 (22–23)
Length of subcephalic setae	15.8 ± 1 (15–17)	19.7 ± 0.6 (19–20)
Buccal cavity depth	25 ± 0 (25–25)	161 ± 13.7 (149–176)
Nerve ring from anterior	144.8 ± 5.6 (141–153)	39.7 ± 4.5 (35–44)
Body diameter at nerve ring	37.3 ± 1.3 (36–39)	46.3 ± 1.5 (45–48)
Pharynx length	417.3 ± 73.5 (314–482)	543.3 ± 3.1 (540–546)
Body diameter at the base of pharynx	40.3 ± 1.5 (39–42)	48 ± 1.7 (47–50)
Anal/cloacal body diameter	33 ± 1.4 (32–35)	39.3 ± 1.5 (38–41)
Spicules length along arc	48.5 ± 4.7 (43–54)	-
Gubernaculum length	7.3 ± 0.5 (7–8)	-
Vulva from anterior	-	1518.7 ± 110.5 (1440–1645)
Body diameter at vulva	-	50.3 ± 1.5 (49–52)
V%	-	0.6 ± 0 (0.5701–0.6186)
Tail length	100.3 ± 4 (97–106)	130.7 ± 10.5 (120–141)
a	51.8 ± 1.8 (50.6–54.5)	48.9 ± 2.4 (46.8–51.5)
b	5.3 ± 1 (4.6–6.8)	4.7 ± 0.4 (4.4–5.1)
c	21.5 ± 0.7 (21–22.4)	19.5 ± 1.5 (18.2–21.1)
c'	3 ± 0.2 (2.8–3.2)	3.3 ± 0.2 (3.1–3.4)

Nicholas and Trueman (2002), Westerman et al. (2022), and Tchesunov et al. (2015), and relationships within Thoracostomopsidae were discussed by Smythe (2015), Jeong et al. (2020), and Tchesunov et al. (2023).

Phylogenetic analysis within family Monhysteridae

Family Monhysteridae was first reviewed systematically by Lorenzen (1978), who identified the holophyly of a single anterior testis and anterior ovary, both positioned to the right of the intestine. Fonseca and Decraemer (2008) recognized 17 genera as valid within Monhysteridae. Hodda (2022) provided a list of 19 genera but omitted the genus *Testudinema*, which is characterized by a buccal cavity with three inner walls and numerous denticles in the stoma. Hodda (2022) reinstated the genera *Litotes* Cobb, 1920, and *Longilaimus* Allgén, 1958, but did not provide further explanation. These two genera should still be considered doubtful, as noted by Gerlach and Riemann (1973/1974). At present, 18 genera should be considered valid.

In the molecular phylogenetic tree, 11 genera—*Diplolaimella* Allgén, 1929, *Diplolaimelloides* Meyl, 1954, *Eumonhystera* Andrássy, 1981, *Gammarinema* Kinne & Gerlach, 1953, *Geomonhystera* Andrássy, 1981, *Halomonhystera* Andrássy, 2006, *Monhystrium* Cobb, 1920, *Monhystera* Bastian, 1865, *Monhystrella* Cobb, 1918, *Tridentula*, and *Thalassomonhystera* Jacobs, 1987—are included. Fifty-eight sequences were selected and analyzed. The SSU-based tree topology clusters into two groups: group A is composed of *Geomonhystera* from terrestrial habitats; group B consists of freshwater, brackish, and marine nematodes.

In clade A, species of *Geomonhystera* form a monophyletic group (100% posterior probability and 100% bootstrap value), acting as a sister group to other monhysterid nematodes. *Monhystera* sp. (PP099580) clusters with the *Geomonhystera* group, which likely indicates that PP099580 was misidentified. According to Andrássy (1981), species of *Monhystera* are exclusively restricted to freshwater environments, whereas *Geomonhystera* species inhabit terrestrial habitats. *Monhystera* sp. (PP099580) was collected from soil in Southern California (Pereira et al. 2024) and should be corrected to *Geomonhystera* based on molecular and habitat evidence.

In clade B1, the genera *Diplolaimella*, *Diplolaimelloides*, *Gammarinema*, *Monhystrium*, and the species *Monhystrella parvella* form a well-supported clade (100% posterior probability and 92% bootstrap value). These genera share morphological similarity in having a buccal cavity with two chambers. Two commensal species, *Gammarinema scyllae* and *Monhystrium mangrovi*, form a well-supported clade (100% posterior probability and 98% bootstrap value). As noted by Westerman et al. (2022), all known species of *Monhystrium* are exclusively found in the gill chambers of land crabs, and eight out of nine known species of *Gammarinema* (only *Gammarinema ampullocauda* is free-living) are associated with crustaceans. These two genera might share a common ancestor associated with crustaceans (Westerman et al. 2022).

In clade B2, *Monhystera*, *Eumonhystera*, and *Tridentula* (*Tridentulus*) form a well-supported clade (100% posterior probability and 100% bootstrap value). These three genera share strong morphological similarities and mainly occur in freshwater. *Tridentula* differs from the other two genera primarily by having three denticles in the buccal cavity. Species of *Eumonhystera* are widely distributed and appear in two different clades, consistent with the tree topology of Westerman et al. (2022) based on SSU sequences.

In clade B3, species of *Halomonhystera* form a well-supported clade (100% posterior probability and 100% bootstrap value). The genus *Halomonhystera* was erected to accommodate marine and brackish species formerly placed in *Geomonhystera* (Andrássy 2006). According to the tree topology, *H. disjuncta* appears in different clades, suggesting high species diversity; the intraspecific p-distance is 0.06. Tchesunov et al. (2015) noted that *H. disjuncta* is a widely distributed species and should be analyzed using both molecular and morphometric methods to identify potential cryptic species.

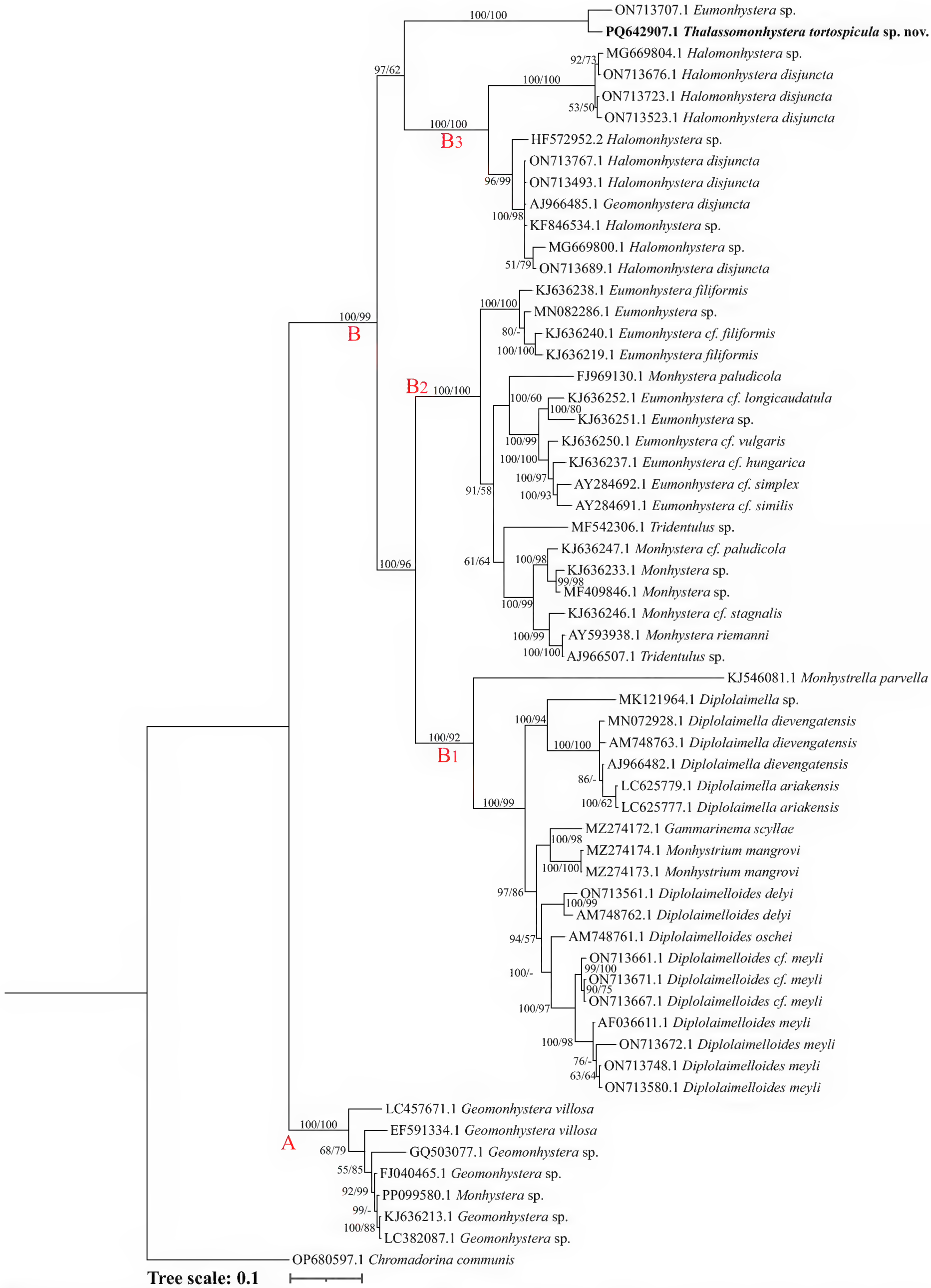

Figure 9. Bayesian inference tree within the family Monhysteridae based on SSU sequences. Posterior probabilities (left) and bootstrap values (right) are shown for the corresponding clades. Sequences obtained in this study are shown in bold. The scale represents substitutions per site.

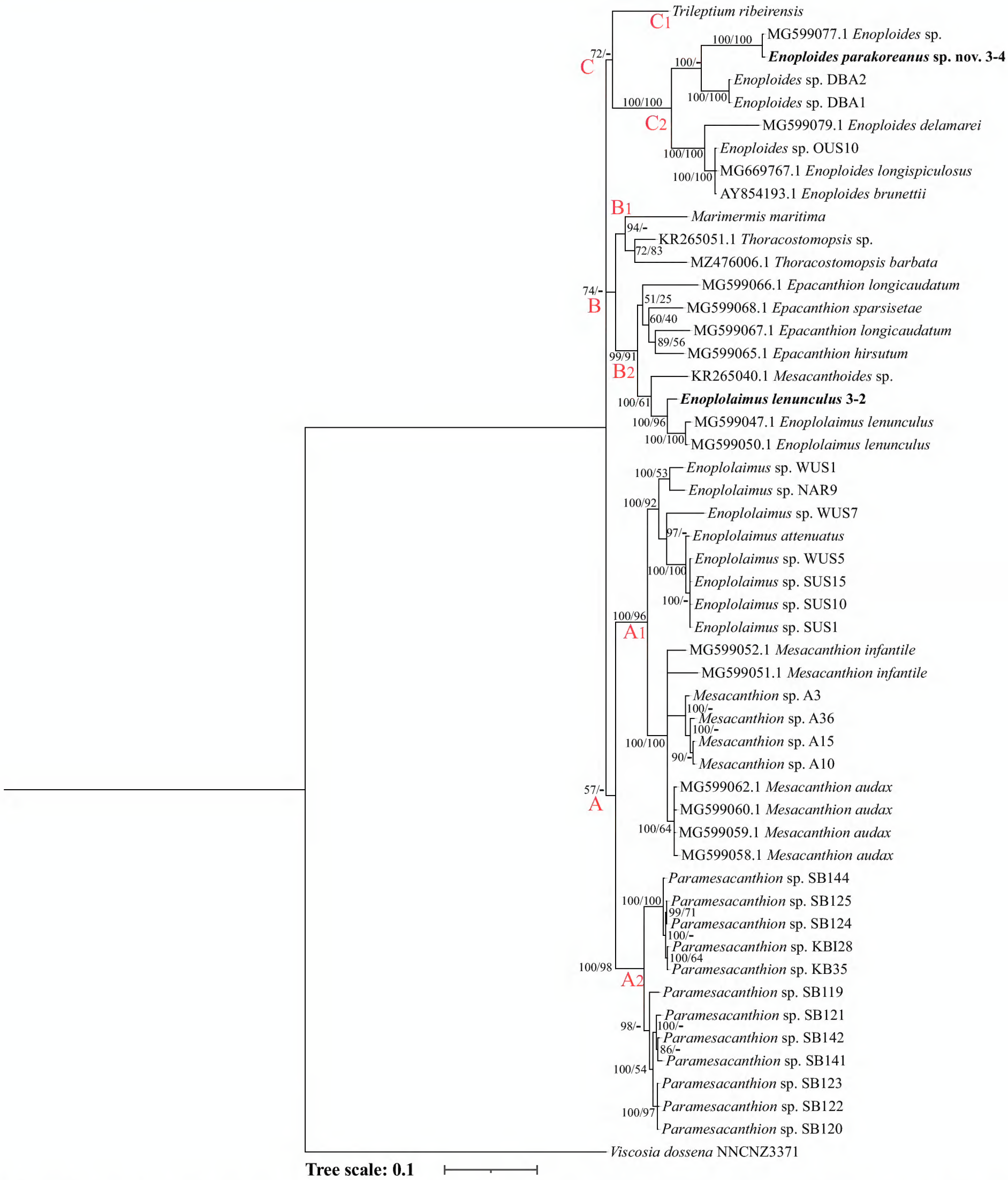

Figure 10. Bayesian inference tree within the family Thoracostomopsidae based on rDNA sequences. Posterior probabilities (left) and bootstrap values (right) are shown for the corresponding clades. Sequences obtained in this study are shown in bold. The scale represents substitutions per site.

Jacobs (1987) divided Monhysteridae into the subfamilies Monhysterinae and Diplolaimellinae, each further divided into two tribes, but this subdivision is not supported by the SSU-based phylogenetic topology, a result also noted by Westerman et al. (2022).

The genera *Geomonhystera*, *Halomonhystera*, and *Monhystera* share high morphological similarity. These genera are mainly differentiated by ecological habitat (Andrássy 2006): *Geomonhystera* is primarily terrestrial, *Halomonhystera* is marine and brackish, and *Monhystera* is freshwater. In the SSU-based topology tree, the three genera form a defined monophyletic clade, which implies that habitat plays an important role alongside morphological characters. Tchesunov et al. (2015) suggested that species delineation in Monhysteridae should be based on an integrative approach combining morphological, molecular, and ecological data.

Phylogenetic analysis within family Thoracostomopsidae

According to Lorenzen (1981, 1994), the family Thoracostomopsidae consists of three subfamilies—Enoplolaiminae, Thoracostomopsinae, and Trileptiinae—with the holapomorphy of dorsolateral orthometanemes with a robust scapulus and no caudal metanemes. The three subfamilies are primarily differentiated by buccal cavity structure: Thoracostomopsinae possesses a long, eversible spear and includes only the genus *Thoracostomopsis* Ditlevsen, 1918; Trileptiinae has three equal-sized teeth and small or absent mandibles, with only the genus *Trileptium* Cobb, 1933; and Enoplolaiminae is characterized by three teeth and three mandibles and contains 14 valid genera (Smol and Coomans 2006; Souza and Maria 2023). In the phylogenetic analysis, three thoracostomopsid subfamilies and eight genera—*Thoracostomopsis*, *Trileptium*, *Enoploides*, *Enoplolaimus*, *Epacanthion* Wieser, 1953, *Paramesacanthion* Wieser, 1953, *Mesacanthoides* Wieser, 1953, and *Mesacanthion* Filipjev, 1927—and one parasitic genus, *Marimermis maritima*, are included.

In clade A, *Paramesacanthion* forms a weakly supported clade with *Enoplolaimus* and *Mesacanthion* (57% posterior probability). These three genera show morphological similarity in possessing well-developed arch-shaped mandibles with claws; the mandibles also consist of two rod-like columns anteriorly united by a curved bar. *Paramesacanthion* differs from *Enoplolaimus* and *Mesacanthion* by the extremely anterior position of the cephalic setae and double-jointed spicules. *Enoplolaimus* and *Mesacanthion* share high morphological similarity and are mainly distinguished by the position of the cephalic setae and the shape of the spicules (Wieser 1953; Jeong et al. 2019). *Mesacanthion* was once considered a subgenus of *Enoplolaimus* and was raised to genus level by De Coninck and Schuurmans Stekhoven (1933) based on differences in dentition, cephalic setae implantation, and the copulatory apparatus and precloacal supplements. This distinction is also supported by the molecular phylogenetic analysis (100% posterior probability and 96% bootstrap value).

In clade B1, the genus *Thoracostomopsis* nests with *Marimermis maritima* and is only weakly supported (94% posterior probability) in the BI analysis, which conforms with the results of Tchesunov et al. (2023). The genus *Thoracostomopsis* is distinguished among free-living nematodes by the autapomorphy of a long eversible spear in the buccal cavity. This spear structure is unique among free-living nematodes and was likely used for piercing food objects, although the diet of the genus remains unknown (Tchesunov et al. 2023). Notably, *Marimermis maritima* shows high affinity with *Thoracostomopsis*, corroborating the findings of Tchesunov et al. (2023). The taxonomic position of *Marimermis maritima*, a marine parasitic nematode, has been debated—originally placed in Enoploida and later transferred to Marimermithida Rubzov, 1980, following the erection of the genus *Marimermis* (Tchesunov 1997). Recent phylogenetic analyses based on rDNA and morphological traits suggest it should be returned to Enoploida, and the autapomorphy of a spear-like structure may also be present in the invasive larvae of *M. maritima* (Tchesunov et al. 2023).

The genus *Epacanthion* has been suggested as a morphological transition between *Enoploides* and *Mesacanthion* based on the lack of solid connections in its mandibular column (Wieser 1953). According to the phylogenetic tree, *Epacanthion* clusters with *Mesacanthoides* and *E. lenunculus* (99% posterior probability and 91% bootstrap value). This result is consistent with Smythe (2015) but differs from Jeong et al. (2020), who reported clustering with *Mesacanthion* in the absence of *Mesacanthoides* and *E. lenunculus*.

Species of the genus *Enoplolaimus* do not form a monophyletic clade. *Enoplolaimus lenunculus* does not cluster with the main *Enoplolaimus* clade, but rather with *Epacanthion* and *Mesacanthoides* (100% posterior probability and 61% bootstrap value). P-distance analysis within *Enoplolaimus* is 0.04, which suggests that the genus may not be monophyletic, as previously proposed by Fadeeva and Zograf (2010).

In clade C, *T. ribeirensis* clusters with *Enoploides* but is only weakly supported in the BI analysis (72% posterior probability). In the ML-based tree topology, *Enoploides* first splits from the main tree clade, and *T. ribeirensis* appears as a paraphyletic clade relative to the other Thoracostomopsidae genera, except *Enoploides*. Six species of *Enoploides* form a well-supported monophyletic clade (100% posterior probability and 100% bootstrap value), consistent with the results of Jeong et al. (2020), Smythe (2015), and Tchesunov et al. (2023) based on SSU analysis. The genus *Enoploides* differs from other genera in Enoplolaiminae by possessing Y-shaped mandibles with claw-like distal ends that curve inward toward the lumen (Jeong et al. 2020), whereas the mandibles of the other three genera in Enoplolaiminae are arch-shaped and composed of two pieces united by an anterior bar (Wieser 1953). *Enoploides parakoreanus* sp. nov. clusters with *E. delamarei*, *E. longispiculosus*, and *E. brunettii* but can be differentiated from them by its short and slightly curved spicules. Morphological and phylogenetic analyses confirm *Enoploides parakoreanus* sp. nov. as a new species.

Advances in genome sequencing technologies, especially the use of whole-genome amplification (WGA), have significantly increased the availability of nematode genome sequences in GenBank. These nematode genomic resources, often analyzed alongside transcriptome data, are primarily used to investigate nematode origins and evolutionary relationships at the order or higher taxonomic levels (Lee et al. 2023; Xue et al. 2025). Ribosomal DNA sequences are widely used in phylogenetic analyses at the family and genus levels. However, extracting rDNA sequences from genomic DNA can be challenging due to assembly difficulties, even when the nematode genome is well assembled. Obtaining rDNA directly via PCR and sequencing with the Sanger method may be much easier and more cost-effective for nematode phylogenetic analysis.

Conclusion

Thalassomonhystera tortospicula sp. nov. is characterized by a medium-sized body, short outer and labial setae, amphidial fovea positioned at the buccal cavity, twisted spicules, gubernaculum with a dorsocaudally directed apophysis, and a conical-cylindrical tail. Phylogenetic relationships based on molecular sequences confirm *Thalassomonhystera tortospicula* sp. nov. as a new species within Monhysteridae. The morphologically similar genera *Geomonhystera*, *Halomonhystera*, and *Monhystera*, which inhabit different environments, are shown in distinct clades, suggesting that ecological factors may play an important role in genus-level delineation.

Enoploides parakoreanus sp. nov. is characterized by a medium-sized body, Y-shaped mandibles with claw-like distal ends, three equally sized teeth, short and slightly curved spicules, a sleeve-shaped gubernaculum enclosing the distal end of the spicules, a tubular-shaped precloacal supplement, and a conical-cylindrical tail. Phylogenetic analysis based on molecular sequences confirms *Enoploides parakoreanus* sp. nov. as a new species within Thoracostomopsidae. Species of *Enoplolaimus* are not monophyletic, and the current phylogeny does not support the division of Thoracostomopsidae into the three subfamilies Enoplolaiminae, Thoracostomopsinae, and Trileptiinae.

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